Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

A STATISTICAL STUDY OF THE STREPTOCOCCI FROM MILK AND FROM THE HUMAN THROAT.*

E. C. STOWELL, C. M. HILLIARD, AND M. J. SCHLESINGER.

(From the Dublin Laboratory, Dublin, New Hampshire.)

INTRODUCTION.

The English and the American work done on the classification of the streptococci by their fermentative properties on carbohydrate and related media has recently been summarized by Winslow.[1] He points out the deficiencies of the English method[2] of recording positive or negative reactions according to the color of litmus-tinted media and proposes the adoption of a uniform method of biometrical study along the lines used by certain American investigators.[3] The fundamental points to be observed are first, the establishing of a low and uniform initial acid reaction of the media to be tested, and second, the quantitative determination of the final acidity, using phenolphthalein as an indicator and titrating with normal sodium hydroxid. The first point mentioned is important on the supposition that acid production by the streptococci is limited only by the acidity of the medium in which they are able to grow and that, approximately, maximum acidity is reached in the time allowed for incubation. Certain tests made by us seem to verify this hypothesis but Broadhurst[4] obtained extraordinarily high results with milk cultures when planted in media of 1.6 per cent initial reaction. Milk was used as an isolating medium and she suggests that "this high acid production" might be considered as due to this fact. It is also a possibility that planting in a medium of high acid reaction may have enhanced the acid-producing properties of the organisms, but for this assumption we have as yet no evidence. The second point is of utmost importance in making the qualitative correlations. I believe, however, that sufficient data are now at hand to warrant an empirical end-point being established, beyond which the reaction shall be recorded as positive fermentation. This point should be established not by any empirical reaction of an indicator to acid but by a study of the results according to biometric principles. Previous work by Winslow seemed to indicate that the streptococci tend to distribute themselves about two modes, the valley or gap occurring at about 0.8–1.0 per cent acid. Reference to the accompanying charts and tables further substantiates the

---

*Received for publication December 20, 1912.

[1] *Jour. Infect. Dis.*, 1912, 10, p. 285.

[2] Gordon, M. H., *Thirty-third Ann. Rept. of Local Govt. Bd.*, 1905; *Rept. on Investigation of Ventilation of Debating Chamber of House of Commons*, 1906; and *Fourteenth Ann. Rept. of Local Govt. Bd.*, 1910–11; Houston, A. C., *Rept. of London County Council*, 1905; Andrews, P. W., and Horder, T. J., *Lancet*, London, 1906, 171, p. 708.

[3] Winslow and Rogers, *Relationships of the Coccaceae*, 1910; Winslow and Palmer, *Jour. Infect. Dis.*, 1910, 8, p. 1; Broadhurst, Jean, *Jour. Infect. Dis.*, 1912, 10, p. 272; Stowell and Hilliard, *Amer. Jour. Dis. of Children*, 1912, 3, p. 287; Morse, M. E., *Jour. Infect. Dis.*, 1912, 11, p. 253; Howe, E. C., *Science*, 1912, 35, p. 225; Rogers and Davis, *ibid.*, p. 230; *Bur. of Animal Ind. Bull. 154*, 1912; Browne, W. W., *Science*, 1912, 35, p. 236.

[4] *Loc. cit.*

previous observations. Winslow has suggested that the division point between acid production and non-acid production "may be placed without serious error at 1.2 per cent normal" acid and in the following discussion we shall use this as the dividing line.

MATERIAL.

We have studied 240 strains of pure cultures of streptococci isolated from 75 separate sources. All cultures used were isolated by one of us so that we know in every case the complete history, age, source, etc., of the organism. Organisms have been isolated from two sources: from cow's milk (55 strains) and from the human throat (185 strains). In accepting more than one strain from a source, we always attempted to take only those showing different cultural or morphological features in the preliminary work of isolation. At times only one culture was obtained from a given source, and at other times as many as five were accepted. We practically never found it impossible to isolate streptococci either from throat swabs or from city milk, suggesting the ubiquitousness of the streptococcus. Fresh milk collected at the dairy or soon after milking was often free from streptococci.

The work has been done in three distinct stages, and hence certain tests have been added in the latter part of the work that were not used in the beginning. For this reason our percentage comparisons that are given in the further discussion are a bit misleading. The following table will assist in clarifying this discrepancy.

TABLE 1.

SHOWING THE NUMBER OF STRAINS USED IN EACH TEST AT THE TWO TEMPERATURES.

The Test	Number Strains Tested		Number Strains not Tested	
	37° C.	20° C.	37° C.	20° C.
Dextrose	240	110	0	130
Lactrose	240	110	0	130
Saccharose	240	110	0	130
Salicin	174	44	66	196
Raffinose	240	110	0	130
Inulin	174	44	66	196
Mannite	189	110	51	130
Morphology	240	...	..	...
Gram Stain	240	...	..	...
Violet positive test	87	...	..	...
Hemolysis	95	...	..	...

It is apparent from this table that the salicin and inulin fermentation tests were added after the work was started, and the violet positive test (first reported by Churchman[1] in July 1912) and hemolysis tests were done in less than half the total number of strains.

METHODS.

Isolation.—The throat specimens were taken on sterile absorbent cotton swabs from the back of the throat. The swabs were then washed off in sterile water tubes and a few drops were immediately plated on nutrient agar made according to standard methods. The milk samples were diluted in sterile water and plated with agar in the usual way. The plates were examined at the end of 36 hours and small, round, white surface colonies, or small, lenticular, subsurface colonies, characteristic of streptococcus growth on this medium, were fished and inoculated into standard nutrient broth. These tubes were examined at the end of 24 hours for growth typical of streptococcus and likely cultures were then examined microscopically in smears stained by Gram's method. Morphology, and purity of culture were thus determined. Cocci occurring three or more in a chain were accepted; diplococci were eliminated. Cocci in pairs within a long chain we admitted. Cultures showing chains containing more than eight elements were classed by us as "long chained." The oval, chained form—*S. lacticus* of Kruse, really of the *B. lactis acidi* group—was never accepted by us. The accepted strains were inoculated from broth into North's medium[2] and were transferred to fresh tubes of North on every third day, until put through the routine tests. Twenty-four-hour-old cultures grown on this gelatin agar medium were always used for the tests and usually they were transferred only once while on this medium before being run through.

Inoculation.—The inoculations into the carbohydrate media to be tested were made with a platinum loop of approximately 4 mm. diameter. Luxuriant growth usually appeared in the water of condensation of the North tube.[3] This was thoroughly mixed, together with whatever surface growth there might be, and then the biconcave drop was lifted with the loop and thoroughly washed off in the carbohydrate broth tube. Duplicate tubes were always inoculated for all tests.

Preparation of media.—Nutrient broth was made in the usual way, using 3 gms. of Liebig's beef extract to the liter. This was made sugar free by inoculating with a pure culture of *B. coli* and incubating. We believe from the experience we have had that this precaution is wholly superfluous when broth is made with the extract, as we obtained only from 0.0 to 0.2 per cent rise in acid after inoculation with coli. To the sugar-free broth, 1 per cent by weight of the test carbohydrate substance was added just before tubing. All test media were sterilized by intermittent sterilization in streaming steam on three successive days. The final reaction of the medium varied from 0.0–0.3 per cent acid.

Incubation.—The duplicate inoculated tubes were incubated at 37° or 20° C., as the case might be, for three days. This time has been found by our own experience and that of others to give approximately maximum acidity with the streptococci.

---

[1] *Jour. Exper. Med.*, 1912, 6, p. 221.

[2] North medium is made with 1 per cent agar and 3 per cent gelatin. It has been suggested by C. E. North for use with pneumococci and streptococci especially. The unusually luxuriant growth of streptococcus and the abundant water of condensation at the base of the slant, from which we could transfer uniform amounts with a loop, made this media especially valuable for our purpose.

[3] Heavier growth may be obtained by adding from a trace to 1 per cent glucose to the North medium.

With the diphtheria group, on the other hand, a longer time has been found necessary for incubation. Two blank tubes were always incubated at the same time for controls.

Titration.—Five c.c. of the inoculated broth were drawn off with a graduated pipette and this amount was drained into a white porcelain evaporating dish, diluted with 45 c.c. of distilled water and titrated cold, against N/20 sodium hydroxid, using phenolphthalein as an indicator. The first indication of permanent pink coloration was taken as the end-point. The pipette was thoroughly washed with distilled water before the next culture was tested. We found it more convenient, toward the end of our work, to run our broth into the test tubes from burettes in carefully measured 5.1 c.c. amounts. When ready for acid determination, the tubes are simply drained into the evaporating dishes. We believe that an accurate amount of culture media in which the organisms are permitted to grow may also give slightly more uniform results.

RESULTS.

Fermentation.—Armstrong[1] says that "The process of fermentation of a sugar is regarded as a series of consecutive reactions each involving simplification of the sugar molecule till it breaks down into carbon dioxid and ethyl alcohol, compounds containing only one and two carbon atoms." The streptococci never carry a fermentation through to the simple elements CO_2 and C_2H_5OH, so that we must make our measurements of the amount of fermentation activity that they exert by determining the amount of acid they split off from the molecule. A chemical molecule must be conceived as a ring of links with here and there a "side chain." As with any combination of links, the strength of the series is represented by the strength of the weakest link, or it is possible that one or more of the "side chains" may be more easily disunited than the others by specific bacterial activity. When the breakdown of the molecule begins, it is always at these weakest points. It is this varying capacity of different bacterial strains to separate the uniting bonds existing between atoms or groups of atoms and thus to avail themselves of the energy liberated in the process, which we try to measure by acid titration.

Metabolic gradient.—Previous work done by two of us on the genus streptococcus and by Howe[2] on the colon group seemed to suggest a very interesting relationship existing between the geometric configuration or the size of the molecule of carbohydrate and the availability of the substance for the organism. Winslow emphasized this "order of relative availability" and pointed out that if "any member of the series is fermented, the chances are that those ahead of it will be fermented also." Howe has aptly

---

[1] *The Simple Carbohydrates and the Glucosides*, p. 53.

[2] *Op. cit.*

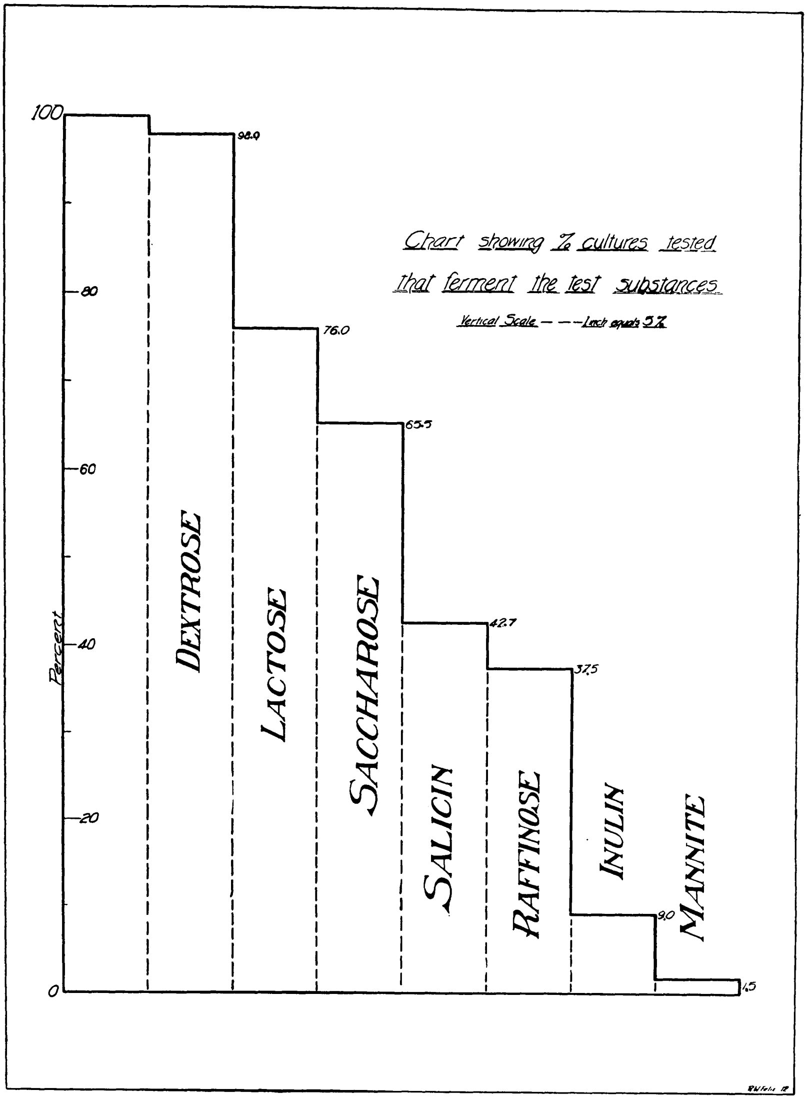

FIG. 1. Showing percentage of cultures that ferment the test substances.

suggested the phrase "metabolic gradient" to represent this phenomenon.

Fig. 1 shows graphically this gradient as found with the organisms and substances used in this study. Winslow in correlating the results of several workers finds the following order to exist:

Substance	Percentage Positive Results
Dextrose	82
Lactose[1]	65
Salicin	75
Saccharose	55
Inulin	32
Mannite	18
Raffinose	12

Our order, it will be observed, is quite different, especially as regards the position of raffinose and salicin in the gradient. These differences are accounted for, first, because of the very small number of saccharose-salicin results used, and second, owing to the large number of faecal cultures included, which ferment mannite and do not ferment raffinose, thus inverting the order of these two substances. Winslow's compilation, being made from the data of a number of workers and from strains isolated from different sources, claims only to be tentative. Our figures have been obtained by a uniform method and the organisms have come from only two sources and we believe that it is wholly logical, as we shall attempt to show.

Table 2 shows the individual correlation of our results arranged as in the Winslow paper referred to. The lower left-hand figure represents what may be termed the aberrant group, i.e., those not reacting in the order with the majority. It will be noted that only in two instances is this group large: in the lactose, saccharose, and raffinose squares and in the salicin-raffinose square. An organism similar to the former group that fails to ferment lactose and yet splits up the higher substances saccharose and raffinose has been described as *S. equinus* by several workers. The group skipping salicin and using raffinos has also been described by Andrews and Horder (see Table 4). Reference to Fig. 1 shows that salicin and raffinose are fermented by about the same number of organisms and the table under discussion shows that if the order were reversed only 20 strains would fall under the bane of aberrant. The order with these two substances is only tentative.

---

[1] Put before salicin because the small number tested on salicin did not justify putting it second.

TABLE 2.

CORRELATIONS OF DIFFERENT ORGANIC MEDIA AS REGARDS THEIR LIABILITY TO FERMENTATION.

	Lactose		Saccharose		Salicin		Raffinose		Inulin		Mannite	
	+	−	+	−	+	−	+	−	+	−	+	−
Dextrose—												
+.......	189	53	166	71	71	99	87	150	15	158	2	181
−.......	0	5	0	5	0	3	0	5	0	3	0	5
Lactose—												
+.......	...	..	146	38	70	74	75	109	13	138	2	128
−.......	...	..	22	36	11	28	22	46	0	39	0	59
Saccharose—												
+.......	...	..	...	..	65	59	84	80	11	113	2	138
−.......	...	..	...	..	6	43	5	73	2	47	0	49
Salicin—												
+.......	...	..	...	..	..	..	53	20	9	62	2	24
−.......	...	..	...	..	..	..	29	71	4	98	0	92
Raffinose—												
+.......	...	..	...	..	..	..	..	...	11	71	1	51
−.......	...	..	...	..	..	..	..	...	2	89	1	136
Inulin—												
+.......	...	..	...	..	..	..	..	...	..	...	1	9
−.......	...	..	...	..	..	..	..	...	..	...	1	111

The accompanying dot chart brings out still more strikingly this food relationship of the streptococci. The position of each strain in its relation to each of the seven substances is represented by a dot (or circle) in the vertical columns. The center of population, as it were, for the substance-acid relationship has been determined and a line has been plotted through these centers (the heavy line). Thus the center of distribution of all the strains when grown in glucose lies at about 3.15 per cent and gradually approaches the zero point with the least available substance, mannite. Note the absence of strains falling between 0.4 and 1.2 per cent, this representing the valley that divides the positive (fermenting) and the negative (non-fermenting) organisms referred to above.

"The order of availability, so far as the streptococci are concerned, corresponds closely to what might be expected from the chemical composition of the substances."[1] We will consider briefly the chemical properties of the substances used to see whether our "metabolic gradient" is logical:

---

[1] Winslow, *loc. cit.*

Dextrose.—A monosaccharid ($C_6H_{12}O_6$) of the formula $CHO.(HCOH)_4CH_2OH$. Glucose shows a great tendency to become further oxidized, evidenced by its activity as a reducing agent. It contains the aldehyd radical. On oxidation it gives rise to gluconic, glucuronic, and saccharic acid.

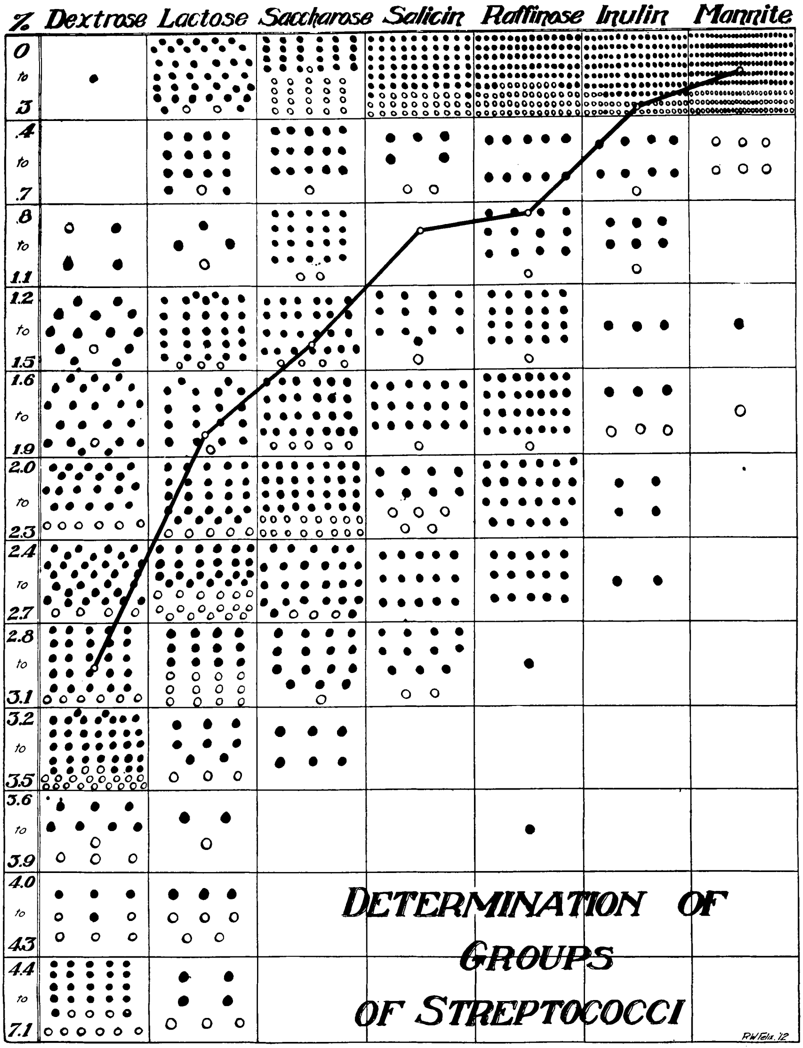

FIG 2. Showing the distribution of the individual strains and the groups into which the milk and throat streptococci fall, according to their capacity to ferment the test substances. The line is drawn through the mathematical center of distribution.

Lactose.—A disaccharid ($C_{12}H_{22}O_{11}$). The stereo-chemical configuration is not definitely decided upon but it is probably made up of a galactose and glucose residue united by a primary alcohol group. The aldehyd group is potentially functional. Upon fermentation it yields, especially, lactic and butyric acid.

Saccharose.—Sucrose or cane sugar is a disaccharid ($C_{12}H_{22}O_{11}$). Fischer's formula pictures it as made up of a glucose and a fructose residue so united as to destroy both the aldehyd and ketone groups, thus giving it only neutral properties. It is readily hydrolized by dilute mineral acids or by the action of the ferment invertase by inversion, yielding a mixture of equal parts of glucose and fructose, invert sugar.

Salicin.—A glucosid of the formula ($C_6H_{11}O_5.O).C_6H_4.OH_2OH$. The glucosids include a large number of substances found in plants having the common property of furnishing glucose and one or more organic residues when hydrolyzed. Salicin is ultimately oxidized to salicylic acid and the acids formed by the oxidation of dextrose.

Raffinose.—One of the commonest trisaccharids ($C_{18}H_{32}O_{16}$). Armstrong gives the following constitutional formula: $C_6H_{11}O_5\text{–}O\text{–}C_6H_{10}O_4\text{–}O\text{–}C_6H_{11}O_5$. It has no reducing properties and behaves chemically very much as sucrose. It is hydrolyzed by mineral acids into melibose and fructose and upon further oxidation may form a variety of substances.

Inulin.—Inulin is a soluble starch compound $(C_6H_{10}O_5)n$. Heated with mineral acids it yields fructose.

Mannite.—A hexahydric alcohol $C_6H_8(OH)_6$ or $CH_2(OH).(CH.OH)_4\ CH_2OH$. It has the usual properties of an alcohol. It is closely related to the sugars yielding levulose on oxidation.

It has been pointed out that with the streptococci the size or bulk of the molecule is the controlling factor and that with the coli the stereo-chemical configuration determines whether the substance shall be available or not.[1] We believe that with the streptococci both factors are important as a study of the summary discussion above and the percentage of organisms that ferment each will tend to show. To determine this metabolic relation more exactly we should work with substances more closely related, e.g., with the disaccharids only as maltose, sucrose, lactose, cellobiose, melibiose, etc.

We may now correlate our fermentation results to determine, if possible, a biometrical classification of the streptococci on this basis. The following table shows that 209 strains (90 per cent) of all the cultures may be classified in nine groups by the use of the seven test substances when incubated at 37° C.

We shall leave the study of the comparative reactions of the throat and milk organisms for a subsequent division of the paper and will discuss only the group reaction. There is a small group—five out of 240—that are wholly incapable of fermenting the most simple substance, dextrose. Still, we must consider it as a specific group as reinoculations proved the organisms capable of growth and limited growth appeared in the sugar tubes, but none, or less than 1.2 per cent acid, was produced. The second group is as yet undescribed so far as we can learn. Many workers have neglected to use glucose as it is so uniformly fermented by the streptococci, and therefore the fact that there is quite an important group with the limited capacity to attack this substance only has been overlooked. Most investigators have used lactose litmus agar for an isolation medium, which has resulted in a selection of forms that at least ferment lactose, and so they have entirely overlooked organisms fermenting a lower substance or with no fermentative properties. We believe that this substance should always be included in the series, as 17 per cent at least will otherwise evade classification or be misplaced.

---

[1] Howe, *op. cit.;* Jackson, *op. cit.*

TABLE 3.

CLASSIFICATION OF THE MILK AND THROAT STREPTOCOCCI ACCORDING TO POSITIVE OR NEGATIVE FERMENTATION OF THE TEST SUBSTANCES AT 37° C.

SOURCE OF CULTURE	NUMBER OF CULTURES	PERCENTAGE		GROUP	DEXTROSE	LACTOSE	SACCHAROSE	SALICIN	RAFFINOSE	INULIN	MANNITE
		Part	Total								
Throat...	5	2.7	2.	I	−	−	−	−	−	−	−
Milk.....	0	0.									
Throat...	27	14.5	12.	II	+	−	−	−	−	−	−
Milk.....	4	7.4									
Throat...	17	9.2	10.6	III	+	+	−	−	−	−	−
Milk.....	13	24.									
Throat...	31	16.7	22.5	IV	+	+	+	−	−	−	−
Milk.....	23	42.5									
Throat...	10	7.5	5.8	V	+	+	+	+	−	−	−
Milk.....	4	10.3									
Throat...	32	23.8	13.3	VI	+	+	+	+	+	−	−
Milk.....	0	0.									
Throat...	9	6.7	3.7	VII	+	+	+	+	+	+	−
Milk.....	0	0.									
Throat...	10	7.5	4.1	VIII	+	−	+	+	+	−	−
Milk.....	0	0.									
Throat...	22	12.	10.	IX	+	+	+	−	+	−	−
Milk.....	2	3.7									

Continuing the examination of the table, we find that more complex substances are successively used by a series of groups through inulin. The large group is the dextrose-lactose-saccharose fermenter. Forty-five per cent of the total number may be classified in groups II, III, and IV. Group V includes a small number—14—because of the similar availability of salicin and raffinose already discussed. Group VI is the second largest, fermenting without skipping a substance, through raffinose. We ought to note in passing that we find not a single milk strain included here. The next group, embracing nine strains of throat streptococci, is the most adaptable of all, and ferments without fail every test substance but mannite. We believe that enough work has been done, on throat organisms at least, to warrant the exclusion of mannite as a test substance in future work. It is invariably a negative substance, the only two instances in which it was positive occurring in aberrant groups.

The following table gives our results in comparison with other classifications of the genus streptococcus by the capacity they show to ferment the Gordon test substances:

TABLE 4.

SHOWING COMPARATIVE CLASSIFICATIONS OF THE STREPTOCOCCI BY THE BIOMETRIC-FERMENTATION METHOD ACCORDING TO THE NUMBER OF SUBSTANCES FERMENTED.

Investigator	Organism	Lactose	Saccharose	Salicin	Raffinose	Inulin	Mannite
Gordon	...	..	..	..	..	..	..
Andrews and Horder	...	..	..	..	..	..	..
Broadhurst	A	−	−	−	−	−	−
Stowell, Hilliard, and Schlesinger	II	−	−	−	−	−	−
Gordon	...	..	..	..	..	..	..
Andrews and Horder	...	..	..	..	..	..	..
Broadhurst	B	−	−	+	−	−	−
Stowell, Hilliard, and Schlesinger	III	+	−	−	−	−	−
Gordon	*1b*, 3, and 4*s*	+	+	−	−	−	−
Andrews and Horder	*S. equinus*	−	+	+	−	−	−
Andrews and Horder	*S. anginosus*	+	+	−	−	−	−
Broadhurst	C	+	−	+.	−	−	−
Stowell, Hilliard, and Schlesinger	IV	+	+	−	−	−	−
Gordon	...	..	..	..	..	..	..
Andrews and Horder	*S. pyogenes, S. mitis*	+	+	+	−	−	−
Broadhurst	D	+	+	+	−	−	−
Stowell, Hilliard, and Schlesinger	V	+	+	+	−	−	−
Gordon	*1a*	+	+	−	+	−	−
Andrews and Horder	*S. salivarius*	+	+	−	+	−	−
Broadhurst	...	..	..	..	..	..	..
Stowell, Hilliard, and Schlesinger	IX	+	+	−	+	−	−
Stowell, Hilliard, and Schlesinger	VIII	−	+	+	+	..	..
Gordon	4*b*	+	+	+	+	−	−
Andrews and Horder	*S. faecalis*	+	+	+	−	−	+
Broadhurst	E	+	+	+	−	+	−
Stowell, Hilliard, and Schlesinger	VI	+	+	+	+	−	−
Gordon	...	..	..	..	..	..	..
Andrews and Horder	...	..	..	..	..	..	..
Broadhurst	F	+	+	+	−	+	+
Stowell, Hilliard, and Schlesinger	VII	+	+	+	+	+	−

The Gordon results (Table 4) show his groupings for organisms isolated from normal throats. The Andrews and Horder are the general groupings shown by a large number of strains from various sources. The Broadhurst figures show the largest groupings appearing in work on 100 milk strains of streptococci.

It will be noted that we have used only six test substances in the above table, omitting dextrose and hence eliminating our first group. This comparison does not show any great uniformity in results and is not at first glance encouraging to the theory that there is a true and stable metabolic gradient. Could great uniformity be expected when we consider the difference in the English and the American methods of obtaining results? The Gordon, and the Andrews and Horder results indicate only whether a litmus-tinted medium, originally faintly alkaline (to litmus), has been reddened or not. It has been found that when results obtained by the quantitative method described above are plotted "they usually show two distinct maxima, one in the neighborhood of the point of no acid formation, the other at a somewhat high acidity."[1] The litmus method would seem to be less accurate than the titration method, and as the end-point of litmus—0.8—is somewhat lower than 1.2 per cent (our standard), we might expect that the English results would be somewhat higher, and this, Winslow has found to be the case. Also, a rough correlation of results might be expected, as the two end-points are fairly close together, and this again seems to be the case.

We must examine this table (Table 4), especially, bearing in mind the source of the organisms used. Gordon's results with throat streptococci agree with ours in two instances: Groups IV and IX. Table 3 shows these groups to contain 16.5 per cent and 12 per cent respectively of our throat strains. The Andrews and Horder results agree with ours in three instances. Their classification is based on the study of about 1,200 strains from various sources. *S. anginosus* corresponds to our group IV, fermenting lactose and saccharose. Our group V checks with two species named by Andrews and Horder: *S. mitis* and *S. pyogenes*. These strains are described as identical in their fermentative properties but vary in their morphology. Broadhurst also checks on this group. The very interesting form, *S. salivarius*, which ferments raffinose and not salicin, has been described by both the English workers. We find only two milk strains classified here and Broadhurst finds none, indicating that this is a specific throat form. The *S. equinus*, which is aberrant to lactose, is described as fermenting only saccharose and salicin. We are inclined to believe that the 10 organisms in group VIII are identical with *S. equinus*, though of course it may be a new form when the origin of these strains is considered. Broadhurst in the work on milk streptococci finds the group II fermenting none of the six sugars used—relation to dextrose unknown—and also V, mentioned above.

---

[1] Winslow, *op. cit.*

Our analysis of the table shows then only three strains that are comparably unaccounted for. Group III ferments lactose only. It is a large group containing representatives from both sources and seems to be definitely limited in its fermentative power. Groups VI and VII are specific throat forms with unusual ability in splitting up the higher substances. They are large enough to be representative and are forms that might logically be expected to be found according to the gradient theory. Our group I is not comparable with other results for reasons previously indicated but we believe it is a group that should not be neglected in the future by the selective methods of isolation previously used by many workers. Groups IV, V, and IX, including about half of the throat organisms classified, represent the only three species described by Andrews and Horder, which, it seems to us, is a very powerful correspondence. We would not expect to find *S. faecalis*.

D. H. Bergey[1] has compared 92 cultures from a variety of sources, using the English method. He finds all of the Andrews and Horder groups except the *faecalis*. There seems to be little correlation between the source from which the organism came and its identity. For example, out of 16 cultures showing the fermenting properties of *S. equinus*, half came from horse manure and eight from the human throat. We are also inclined to question the capacity of *S. mitis* to ferment inulin and its failure to ferment saccharose; and of *equinus* to ferment salicin, according to the original intentions of Andrews and Horder. *Salivarius* and *anginosus* are grouped together by Bergey but we believe that a general negative raffinose result with the latter organism is now established. On the whole, however, the work substantiates the food relationships of the streptococci when studied by the biometric method.

---

[1] *Jour. Med. Research*, 1912, 27, p. 67.

When the broths are incubated at the room temperature we find a very marked change in the gross grouping of the streptococci in their relation to the test substances. We find that 89 out of the 110 strains studied, or about 81 per cent, may be grouped around four reactions in sugars. The following table shows this relationship. Two throat strains only show the capacity to ferment any substance other than a single sugar when incubated at 20° C.

TABLE 5.

Classification of Milk and Throat Streptococci According to Positive or Negative Fermentation of the Test Substances at 20° C.

Source of Culture	Number of Cultures	Percentage		Group	Dextrose	Lactose	Saccharose	Salicin	Raffinose	Inulin	Mannite
		Part	Total								
Throat...	38	64.5	35.4	I*a*	–	–	–	–	–	–	–
Milk.....	1	2.0									
Throat...	14	23.9	17.2	II*a*	+	–	–	–	–	–	–
Milk.....	5	10.									
Throat...	1	1.7	15.4	III*a*	+	+	–	–	–	–	–
Milk.....	16	32.									
Throat...	1	1.7	12.7	IV*a*	+	+	+	–	–	–	–
Milk.....	13	26.									

The importance of this table does not lie so much in the groups shown as in the striking differentiation between the organisms from the two sources, throat and milk. The capacity to ferment any sugar other than dextrose seems to be wholly denied streptococci from the throat while 56 per cent of all the strains isolated from milk flourish on lactose media.

COMPARISON OF THE MILK AND THE THROAT STREPTOCOCCI.

This leads us to a brief consideration of differential fermentative features of the streptococci from the two sources, cow's milk and the human throat. The streptococci isolated from milk were taken in part from fresh milk collected at the dairy or from the milk wagon on a short country route, and from samples of dirty city milk.

Reference to Tables 3 and 5 shows that there are certain groups that are exclusive of either milk or throat organisms. Thus at

37° C. groups VI, VII, and VIII—using substances beyond salicin—contain no milk streptococci at all. So far as our work is concerned we may call these, distinctly throat groups. At 37° C. group IV is most specific for milk strains, containing 42 per cent of the total. Table 3 shows that groups III and IV are distinctly milk groups.

In a previous paper of ours we have noted the fact that "throat streptococci do not readily ferment at 20° any of the sugars used, while the milk organisms attack the same sugars and to the same extent at this temperature as at 37° C." Our further studies have shown this preliminary conclusion still to be the most striking differential feature. Reference to the accompanying curves shows very positively this difference in temperature-fermentation relation.

Reference to Fig. 2 or to the classification tables will show this same thing in a different way. It is very obvious that the throat streptococci are less fastidious as to the substance they ferment but are very susceptible to temperature relations, while the milk strains very seldom give a positive acid reaction when grown in the presence of a substance higher in the series than saccharose but ferment through this substance equally well at either 20° or 37° C.

The reactions are so constant and so different between the strains from the two sources that we feel justified in suggesting certain empirical standards for identifying the original source of unknown strains. We believe that the following features are sufficient to separate milk from the throat streptococci: (1) they yield over 2.5 per cent acid in lactose and saccharose at 37° C.; (2) they seldom ferment a substance higher than saccharose in the metabolic series; (3) they readily grow in dextrose, lactose, and saccharose at 20° C. Throat streptococci, on the other hand, (1) seldom yield over 2.5 per cent acid in any substance at any temperature; (2) over 40 per cent yield over 1.2 per cent acid in either salicin or raffinose or in both at 37° C.; (3) at 20° C. they almost never attack any of the test substances.

Reduced to still lower terms we would say that six different tubes of media would place an unknown streptococcus if it came from one of the sources studied: dextrose, lactose, raffinose, and

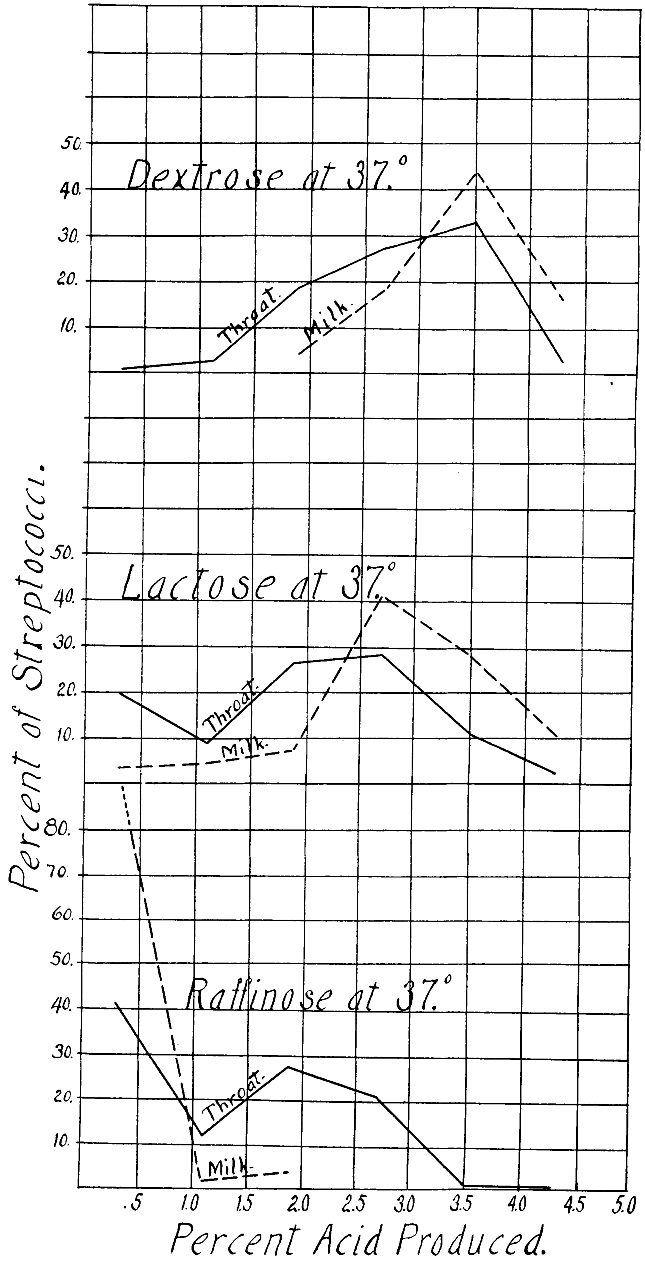

FIG. 3.—Curves of dextrose, lactose, and raffinose, showing the percentage of cultures from each source in relation to the percentage of acid formed when grown at 37° C.

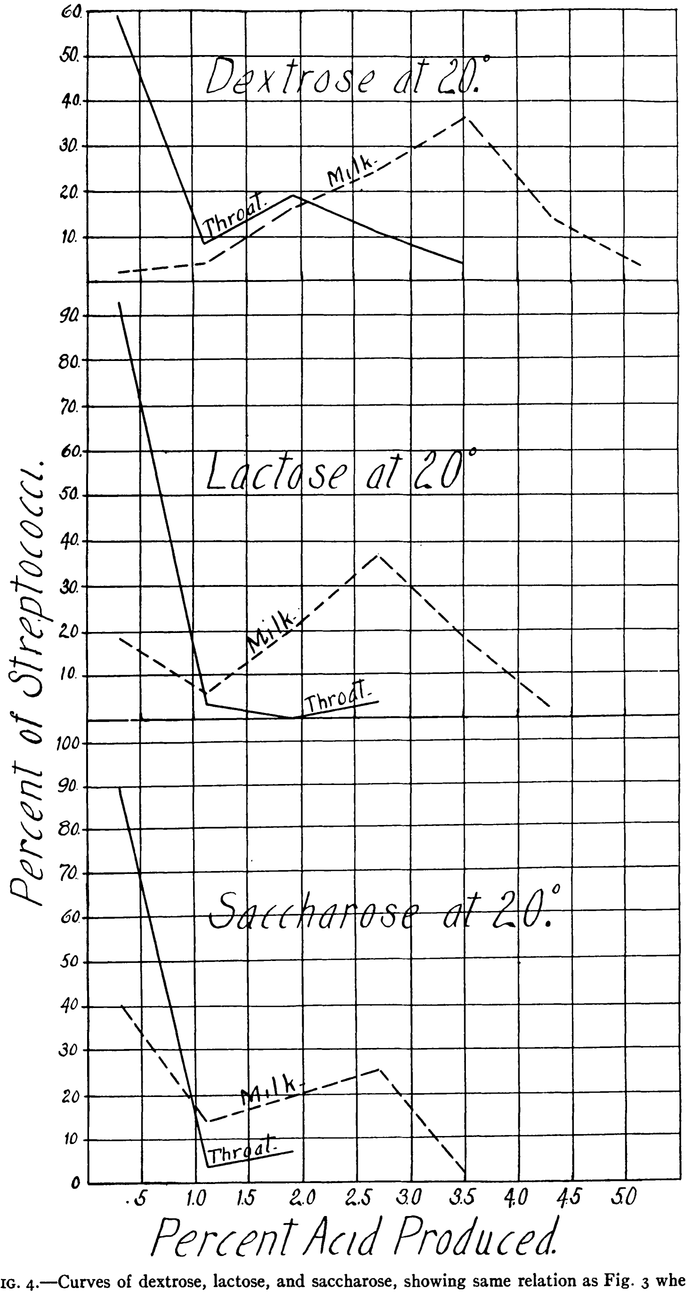

FIG. 4.—Curves of dextrose, lactose, and saccharose, showing same relation as Fig. 3 when grown at 20° C.

salicin at 37° C., lactose and saccharose at 20° C. With a milk strain we expect above 2.5 per cent acid in dextrose and lactose at 37° C.; negative fermentation in the other two media at that temperature; fermentation in the two substances incubated at 20° C. A strain from the throat would be expected to give lower acid production in all tests, should ferment salicin or raffinose, and should give little or no acid when incubated at 20° C. in the two substances suggested. Four of the fixed tests would be considered diagnostic.

FEATURES OTHER THAN FERMENTATION STUDIED.

Morphology.—We examined most of our cultures twice microscopically to obtain, if possible, some correlation between the length of chain, size of the coccus, etc., and other features. We were unable to group these with any other characteristics. The only observation worthy of note is that only about 25 per cent of all the throat cultures were long chained, i.e., 10 cells or more, while about 75 per cent of the milk organisms were grouped by us as "long."

Gram stain.—We carefully stained 24-hour cultures on North media by the Gram method in the hope that this reaction might prove of diagnostic value. We found that we could make no correlation with this stain reaction and any other characteristic. The large majority of the cultures retained the stain, a few stained irregularly, and a few were wholly decolorized.

Violet positive test.—Churchman recently reported a study of the "bacteriostatic" properties of gentian violet when added to media in minute doses. He found that there was a very consistent parallelism between this inhibiting property of the stain in media and the Gram stain. Following his technic of divided plate, we tested 87 of our cultures. All but five strains refused to grow on the violet side of the plate while all grew well on the plain agar control side. Included in those that were "violet positive," i.e., those that did not grow, were several gram-negative strains. The parallelism with the coccaceae is apparently not absolute, as is pointed out by Churchman. The test is of no differential value in this study.

Hemolysis.—The hemolytic property of 92 strains was studied in the following manner: A few drops of fresh, sterile rabbit's blood were added to cooled melted agar tubes and petri plates were then poured. After incubation of the plates for 24 hours, freshly isolated cultures were streaked on the plate with a small, light, platinum loop. The plates were examined after 24 hours at 37° C. and if there was any hemolysis the clear area was measured and recorded in millimeters. The plates were usually kept for a longer period to see if any further changes took place, but our records were kept on the 24-hour reading.

Of the 92 strains so tested, 17 gave hemolysis. The breadth decolorized varied in extent from 0.5–2.5 mm. Five of the 17 giving a positive hemolytic test were from "normal" milk, five—the most vigorous—from milk of dairies where udder trouble was known to exist, and seven were from normal throats. In three instances the "greenish" color described by Ruediger[1] as typical of the *S. lacticus* was noted. The capacity to hemolyze rabbit's blood during the short incubation period allowed is not correlated with any other of the characteristics studied, so we have neglected it in our groupings. As a single cultural feature to be studied in connection with pathogenicity, it is invaluable, as has been shown by other workers.[2]

Character and amount of growth.—The character and the luxuriance of growth on North medium and in broth have been studied by us but we have been able to make no correlations worthy of discussion. The length of chain and consistency and cloudiness of the broth are correlated. In the test broths the amount of growth varied almost directly with the degree of acidification. We may note here that zero acid indicates no growth almost invariably. In other words, the substances which are not fermented act as inhibiting or "bacteriostatic" agents and are non-available for use. It is possible that in certain instances it acts even as a germicidal agent, though we did not make any extended research into the question. Reinoculations after the three days' incubation frequently found the tubes sterile but doubtless the bacteria, being unable to reproduce, had died from old age in this time. We are positive that no growth did not mean failure to inoculate the tubes, as our duplicates always checked with remarkable constancy and in most instances where they did not check, i.e., growth in one tube and none in the other, we confirmed the test by reinoculating in duplicate a second time.

---

[1] *Op. cit.*

[2] Jupille, F., *Ann. de L'Inst. Past.*, 1911, 25, p. 918; Ruediger, G. F., *Amer. Jour. Pub. Health*, 1912, 2, p. 107; Davis, D. J., *Jour. Amer. Med. Assoc.*, 1912, 58, p. 1851.

Stability of the fermentation tests.—Frequently, where we were doubtful of a result, we inoculated fresh tubes a week or even longer after the first series had been run. Such tests usually confirmed our first finding. At one time we ran 72 strains through raffinose several weeks apart and the reactions checked closely. The first 50 cultures we worked with were not of uniform age when passed through the various test substances and we ran them all through a second time several weeks later. (A few of the cultures had died in the interim.) With hardly an exception they confirmed the first records. We believe, therefore, that the fermentative properties of the streptococci, exclusive of pathogens, are reasonably stable when kept under cultivation and hence may be depended upon to remain constant in their normal environment. Rogers and Davis[1] have recently noted the same fact. This feature, of course, lends value to our general thesis that the fermentative tests may be depended upon as of diagnostic value.

SUMMARY AND CONCLUSIONS.

Two hundred and forty pure strains of streptococci isolated from milk and from the normal human throat have been compared as to their morphology, gram stain reaction, character, and amount of growth, and their quantitative acid production in seven carbohydrate and related organic substances. Hemolysis and the gentian violet cultural test have been studied in part of the cultures.

We have not found that any of the features other than the fermentative reactions are sufficiently correlated with one another or with acid production to assist in the fixing of the organisms in groups or in distinguishing the source of isolation.

The seven organic substances tested show a definite order of availability for acid production. This order and the percentage of cultures yielding 1.2 per cent or more of acid when grown at 37° C. for three days is shown in the following table:

---

[1] *Bur. of Animal Ind. Bull. 154*, 1912.

Glucose (monosaccharid)	98 per cent
Lactose (disaccharid)	76 per cent
Saccharose (disaccharid)	65.5 per cent
Salicin (glucosid)	42.7 per cent
Raffinose (trisaccharid)	37.5 per cent
Inulin (starch)	9.0 per cent
Mannite (an alcohol)	1.5 per cent

According to the positive reaction—over 1.2 per cent acid—in the test substances, 90 per cent of the cultures may be correlated so as to fall into nine groups.

Milk streptococci are distinguishable from the throat organisms of the same morphology (1) by their higher acid production in substances in which they grow, (2) by their greater independence of temperature relations, (3) by their general incapacity to ferment more complex test substances than the disaccharids. On the other hand, the throat strains in at least half the cases (1) ferment a more complex test substance and (2) in almost all cases fail to ferment a higher test substance than the monosaccharid at the room temperature.

We do not attempt to fix names or to establish any ultimate taxonomic relations by this study. We feel that the study, together with contemporary work in bacteriological biometrics, indicates the most fruitful, though laborious, way in which we are obliged to systematically split up our present unwieldy and meaningless genera.

In concluding, the authors wish to express their great appreciation of the hearty co-operation and skilful assistance of Marjorie H. Boyce, assistant in the Dublin Laboratory for 1912.